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REMARKS

Claim 16 now recites that dicotyledonous plant cells or dicotyledonous plant explants are transformed with *Agrobacterium rhizogenes*. Support is found in the specification notably on page 3 lines 27-28, page 11 lines 28-29, and page 14 lines 17-18.

Claim 16 is also amended to recite that the transformation step of (a1) or (a2) induces the formation of roots on the transformants. Support is found on page 2, line 19.

Claim 17 has been amended to recite that that the formation of roots in step a1) or a2) occurs on the plant cells or plant explants and that the transformants are the roots.

Claim 25 has been amended to include petioles, support for which can be found on Page 7, line 32.

No new matter has been introduced into the application by these amendments.

Election / Restriction:

Applicant again traverses the restriction requirement, and solicits favourable reconsideration of the requirement in the light of the following comments.

Applicant respectfully submits that the special technical feature common to all the claims has not been considered. It is stated on page 1, lines 7-11, of the specification that "[t]he subject of the present invention is a method for obtaining transgenic plants, characterized in that it uses transformation by *Agrobacterium rhizogenes* **combined** with VISUAL sorting of the transformation events, based on coloring, which is simple to carry out and is rapid, of the transformed roots expressing the transgene". (emphasis added).

Thus, the special technical feature of the invention is the combination of **both transformation by *Agrobacterium rhizogenes*** AND **visual sorting** of the transformation

events. This special combination is novel and common to all of the present claims.

WO 94/13790 does not teach this special combination, nor the use of a visual sorting of transformants based on coloring.

In view of the above arguments, and those made in Applicant's Response to Restriction Requirement, filed on June 25, 2003, Applicant strongly urges favourable reconsideration and withdrawal of the restriction requirement.

Claim Rejections:

The rejection of claims 16-27, 30-32, and 36-37 under 35 U.S.C. 112, second paragraph, is obviated in part by appropriate amendment, and traversed in part.

Applicant will address each issue under 35 U.S.C. 112, second paragraph, in the order in which they appear in the Office Action.

Claim 16:

Claim 16 has been amended to make clear that the method relates to two alternatives: the first alternative being steps a1) and b) and c) and d), and the second alternative being a2) and b) and c) and d).

Applicant believes that the term "gene encoding a protein producing H_2O_2" is clear to one of ordinary skill in the art. A gene that "encodes" a protein includes a coding sequence for that protein, in this case a protein producing hydrogen peroxide.

In Claim 16, the term "in a context allowing its expression" in (a1) has been replaced with "wherein said gene is flanked by elements necessary for expression of the gene", basis for which is found in the specification on page 4 lines 28 to 31. A similar amendment has been made to the language of step (a2), basis for which is found in the specification on page

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9 lines 27 to 28.

Claim 16 has been amended to indicate that the test is carried out in the presence of a substrate for the protein with H_2O_2 producing activity, and peroxidase for revealing the formation of H_2O_2. Support for this amendment is found, *inter alia*, on page 3 lines 5-10, of the specification). Applicant submits that the peroxidase-based colorimetric test is a test that indicates the presence or absence of a protein with H_2O_2 producing activity. In the alternative (a1) of claim 16, if a change of color is visualized when carrying out this test it may then be concluded that the protein with H_2O_2 producing activity has been integrated (by transformation) into the plant. In the alternative (a2) of claim 16, if a change of color is visualized when carrying out this test it may then be concluded that both the protein with H_2O_2 producing activity and the protein of interest have been integrated (by transformation) into the plant, as these two proteins are comprised into the same recombinant DNA (see also page 7 lines 24-30, of the specification).

In claim 16, step (c), it is now specified that the transformants are those which express the H_2O_2 producing protein.

In claim 16, step (c), it is now specified that "transgenic plants" are regenerated out of the selected transformants, support for which can be found in the specification on page 10, line 3.

The term "roots" no longer appears in claim 16, step (c), rendering moot the objection for lack of antecedent basis for this term.

In claim 6, step (c), it is now specified that the expression of the H_2O_2 producing protein is monitored by a peroxidase-based colorimetric test (and not the plantlets obtained)

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(see specification on page 7, lines 24-30).

In claim 16, step (d), the term "phenotype" has been withdrawn. The plants which contain pRi (one of the two vectors used for *Agrobacterium rhizogenes* transformation: see specification page 4 line 2-10 and page 13 lines 18-21) exhibit, in particular, crinkled leaves and shorter internodes.

Applicant respectfully disagrees with the Examiner's conclusion that claim 16 is indefinite as incomplete in failing to recite a final step of recovering the desired product. Claim 16 is directed to a method for obtaining transgenic plants. It is clear from step (d) that the final product is a transgenic plant containing the transgene according to (a1) or (a2). The sorting according to step (d) of new claim 38 allows the selection or the confirmation of transgenic plants. Favourable reconsideration is urged.

Claim 18:

Claim 18 now specifies that the colorimetric test in step (b) is carried out on liquid incubation medium after decontamination of the transformed plant cells or plant explants, wherein the decontamination comprises eliminating the Agrobacteria from the liquid medium. Support for the amendment can be found in example 4-1 on page 20 of the specification.

Claim 19:

Claim 19 has been cancelled, rendering moot the objections raised to this claim.

Claims 20 and 21:

Applicant submits that the term "substrate" in claims 20 and 21 is clear to a person of ordinary skill in the art. Claim 16 now recites that the substrate is a substrate for the protein

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with H_2O_2 producing activity. Also, claim 20 now recites that the oxidation of the substrate is accompanied by a change of color. Support is found in the specification on page 3, line 9, and on page 12 lines 19-29.

According to the present invention, many substrates could be used (see specification page 12 lines 19-29) depending on the choice of the protein with H_2O_2 producing activity.

The goal is that when the protein with H_2O_2 producing activity is expressed in the transformed plants, and in the presence of the substrate, a change in color permits the visual detection of transformation occurrence.

When the oxalate oxidase is used as protein producing H_2O_2 activity, a blue color is obtained on the transformed parts of the plant in presence of the substrate and peroxidase.

Claim 23:

Applicant submits that it is clear from the specification on page 8, lines 23-26, that the late stage of development relates to the late stage of development of the plant, and the gene of interest may be expressed in the plant after plant transformation. It is therefore respectfully submitted that the term "late stage of development" in claim 23 is clearly understood by a person of ordinary skill in the art.

Claim 25:

A "scape" is defined in the Chambers Science and Technology Dictionary as "the flowering stem, nearly or quite leafless, arising from a rosette of leaves and bearing a flower, several flowers or a crowded inflorescence; e.g. the dandelion." (see attachment).

On the internet web-site http://dict.die.net/scape/, scape has the following definition: "erect leafless flower stalk growing directly from the ground as in a tulip (syn=flower stalk).

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In view of the above amendments are remarks, Applicants respectfully submit that the amended claims are definite within the meaning of 35 U.S.C. 112, second paragraph. Withdrawal of this ground of rejection is therefore respectfully requested.

Claims 16-27, 30-32, and 36-37 were rejected under 35 U.S.C. 112, first paragraph, as containing subject matter not described in the specification in such a way to enable the invention.

The rejection is obviated in part by appropriate amendment, and traversed in part.

Applicant will address each issue under 35 U.S.C. 112, first paragraph in the order in which they appear in the Office Action.

"Any plant":

The rejection indicates that the disclosure is not enabling for any plant. Claim 16 now recites that dicotyledonous plants or dicotyledonous plant explants are transformed with *Agrobacterium rhizogenes*.

"A protein producing hydrogen peroxide":

The rejection indicates that the disclosure is not enabling for a "protein producing hydrogen peroxide". Claim 16 now recites that the peroxidase-based colorimetric test is carried out in the presence of a substrate for the protein with H_2O_2 producing activity.

"Transforming with *Agrobacterium rhizogenes* containing a vector":

The rejection indicates that the disclosure is not enabling for the transformation of plants with Agrobacterium that does not contain a T-DNA. The rejection also indicates that the disclosure is not enabling for the transformation of plants using Agrobacterium unless the genes are flanked by appropriate T-DNA sequences.

Claim 16 now recites that the vector carries a T-DNA comprising a gene encoding a protein producing H_2O_2.

"Sorting according to phenotype":

In the light of the amendment to step (d) of claim 16, it is believed that this rejection is now moot.

"Protein of interest conferring resistance to disease caused by . . .":

The rejection indicates that the disclosure is not enabling for pathogenic diseases caused by any fungi, any bacteria, any arthropod, and nematode, and where the protein of interest is of agronomic or industrial interest. Applicant submits that a person of ordinary skill in the art would appreciate that any gene of interest can be expressed using the method of the invention. For instance, page 8 line 29 to page 9 line 23 of the specification provides numerous examples of genes of interest have been described, some of which have also been exemplified.

The genes of interest described in the present specification are not a limitation. The method according to the present invention is a specific method of transformation but the specificity is not provided by the choice of the gene of interest. So any gene of interest is suitable.

In view of the above, Applicant respectfully submits that one of ordinary skill in the are is able to practice the claimed invention without an undue amount of experimentation. Accordingly, withdrawal of the rejection under 35 U.S.C. 112, first paragraph, is urged.

The rejection of claims 16, 19, 24, 25, 30, 32, and 37 under 35 U.S.C. 103(a) over Simpson et al. et al. in view of Zhang et al. is respectfully traversed.

The inventiveness of the presently claimed method is based on the **combination** of

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plant cell transformation by ***Agrobacterium rhizogenes*** and **visual sorting** of the transformation events, **based on colouring**. The advantages of this combination method are that the method is simple to carry out and is rapid.

Such a method is nowhere disclosed or suggested in the prior art of record.

Simpson et al. teaches a method for transforming plants comprising transforming tobacco cells with *Agrobacterium rhizogenes*. Notably, however, Simpson et al. does not teach the use of a gene encoding a protein producing H_2O_2, nor a selection step by a peroxidase-based colorimetric test wherein the test is carried out in the presence of a substrate for the protein with H_2O_2 producing activity and peroxidase for revealing the formation of H_2O_2.

As such, the method of Applicant's invention is not *prima facie* obvious from this reference.

The Zhang et al. reference does not cure the basic deficiencies of the Simpson et al. reference.

Zhang et al. reports increased activity of the germin-like oxalate oxidase in association with the response of barley to powdery mildew fungus, *Erysiphe graminis f.sp. hordei.* The increase is detected in a colorimetric assay as well as on activity blots using extracts of both resistant and susceptible leaves (see summary page 139 column 1).

Notably, Figure 1 on page 141 of Zhang et al. is illustrates the oxalate oxidase activity in response to pathogen attack. The increase of a specific H_2O_2-producing plant enzyme activity following pathogen attack is also discussed on page 142 column 2. Furthermore, samples of barley isogenic lines were analyzed (see the legend to Figure 1 on

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page 141).

It is well known that oxalate oxidase is a pathogen resistance gene. However, Zhang et al. does not teach a method for transforming plants with *Agraobacterium rhizogenes*. Moreover, Zhang does not teach the use of gene encoding a protein producing H_2O_2 as a visual selectable marker.

Accordingly, motivation does not exist for a person of ordinary skill in the art to combine the teachings of Simpson et al. and Zhang et al., because Zhang et al. does not relate to the selection step of a transformation method but, rather, to pathogen resistance analysis. The teachings of these two documents are so far removed to consider such a combination.

Based on the foregoing, Applicant asserts that the Simpson et al. and Zhang et al. al. references, when taken as a whole for what they individually and in combination reasonably teach one of skill in this art, do not disclose or render the claimed invention obvious. Applicant's choice of claim limitations has been determined from what has solved the problem before them, and there is simply no motivation to modify or choose from Simpson et al. and Zhang et al. al..

In view of the deficiencies in the art, none of the present claims are *prima facie* obvious, and, accordingly, withdrawal of the rejections under 35 U.S.C. 103(a) is respectfully requested.

Applicant respectfully submits that the present invention is now in condition for allowance. Early notification to that effect is earnestly solicited. If any final points remain that can be clarified by telephone, Examiner Helmer is encouraged to contact Applicant's attorney at the number indicated below.

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Applicants hereby petition the Commissioner for Patents to extend the time for reply to the notice dated October 23, 2003, for three (3) months from January 23, 2004 to April 23, 2004. A duly completed credit card authorization form is attached to effect payment of the extension fee.

Respectfully submitted

DENNISON, SCHULTZ,
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April 23, 2004

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"CLEAN" VERSION OF CLAIMS

16. A method for obtaining transgenic plants, comprising

(a1) transforming dicotyledonous plant cells or dicotyledonous plant explants with *Agrobacterium rhizogenes* containing a vector carrying a T-DNA comprising a gene encoding an H_2O_2 producing protein, wherein said gene is flanked by elements necessary for expression of said gene; and wherein said transformation induces the formation of roots on the transformants;

or

(a2) transforming dicotyledonous plant cells or dicotyledonous plant explants with *Agrobacterium rhizogenes* containing a recombinant DNA comprising both a gene encoding an H_2O_2 producing protein and a gene encoding a protein of interest, wherein said gene encoding the H_2O_2 producing protein and said gene encoding a protein of interest are flanked by elements necessary for expression of said genes; and wherein said transformation induces the formation of roots on the transformants;

and

(b) selecting the transformants which express said H_2O_2 producing protein by a peroxidase-based colorimetric test, wherein said test is carried out in the presence of a substrate for said protein with H_2O_2 producing activity and peroxidase for revealing the formation of H_2O_2;

and

(c) regenerating transgenic plants out of the selected transformants and monitoring the expression of said H_2O_2 producing protein within the plantlets obtained, wherein said expression is monitored by a peroxidase-based colorimetric test;

and

(d) sorting the plantlets which do not contain the T-DNA of pRi of *Agrobacterium rhizogenes* and optionally carrying out a molecular analysis of the progeny of said sorted plants, allowing the selection or the confirmation of the obtainment of transgenic plants only containing the transgene and not the T-DNA of pRi of Agrobacterium rhizogenes.

17. The method of claim 16, wherein the transformation according to step (a1) or (a2) induces the formation of roots on the plant cells or plant explants;

and wherein

step (b) comprises selecting the roots which express said H_2O_2 producing protein by a peroxidase-based colorimetric test, wherein said test is carried out in the presence of a substrate for said protein with H_2O_2 producing activity and peroxidase for revealing the formation of H_2O_2;

and

step (c) comprises regenerating transgenic plants out of the selected roots and monitoring the expression of said H_2O_2 producing protein within the plantlets obtained, wherein said expression is monitored by a peroxidase-based colorimetric test;

and

step (d) comprises sorting the plantlets which do not contain the T-DNA of pRi of *Agrobacterium rhizogenes* and optionally carrying out a molecular analysis of the progeny of said sorted plants, allowing the selection or the confirmation of the obtainment of transgenic plants only containing the transgene and not the T-DNA of pRi of *Agrobacterium rhizogenes*.

18. The method according to claim 16, wherein said colorimetric test in step (b) is carried out on liquid incubation medium after decontamination of the transformed plant cells or plant explants, wherein said decontamination comprises eliminating agrobacteria from said liquid medium.

Claim 19 canceled.

20. The method according to claim 16, wherein said selection in step (b) is carried out in the presence of a saturating concentration of said substrate for said H_2O_2 producing protein, wherein oxidation of said substrate is accompanied by a change of color.

21. The method according to claim 20, wherein the saturating concentration of substrate is from 5 to 50 mM.

22. The method according to claim 16, wherein the colorimetric test in step (c) is carried out on a sample of plant tissue from the plantlets obtained.

23. The method according to claim 16, wherein the gene of interest is a gene of interest which is expressed at a late stage of development of the plant.

24. The method according to claim 16, wherein the plant cells are plant cells obtained from a member selected from the group consisting of rape, cauliflower, sunflower, tomato, and tobacco.

25. The method according to claim 16, wherein the plant cells are cells of the cotyledons, hypocotyls, petioles, or floral scapes.

26. The method according to claim 16, wherein the plant cells do not endogenously produce oxalate oxidase.

27. The method according to claim 16, wherein the protein of interest is an endochitinase.

Claims 28 and 29 canceled.

30. The method of claim 16, further comprising expressing and purifying the protein of interest.

31. The method of claim 16, wherein the plant cells are transformed using an expression vector comprising a promoter, wherein said promoter is selected from the group consisting of the Cauliflower Mosaic Virus (CaMV) 35S promoter, the superpromoter chimeric promoter SPP, the rice actin promoter, the barley HMGW promoter, the PCRU radish cruciferin gene promoter, the corn γ-zein gene promoter, the *Arabidopsis* PGEA1 promoter and the *Arabidopsis* PGEA6 promoter.

32. The method of claim 16, wherein the expression of the gene encoding a protein of interest confers resistance to disease caused by an organism selected from the group consisting of fungi, bacteria, arthropods and nematodes.

Claims 33, 34, and 35 canceled.

36. The method of claim 16, wherein the gene encoding a protein of interest encodes a protein of agronomic or industrial interest.

37. The method of claim 16, wherein the gene encoding a protein of interest encodes a protein conferring resistance to pathogenic agents.